Bevacizumab and temozolomide in patients with first recurrence of WHO grade II and III glioma, without 1p/19q co-deletion (TAVAREC): a randomised controlled phase 2 EORTC trial

van den Bent, Martin J ; Klein, Martin ; Smits, Marion ; et al ; Weller, Michael

Abstract: BACKGROUND Bevacizumab is frequently used in the treatment of recurrent WHO grade II and III glioma, but without supporting evidence from randomised trials. Therefore, we assessed the use of bevacizumab in patients with first recurrence of grade II or III glioma who did not have 1p/19q co-deletion. METHODS The TAVAREC trial was a randomised, open-label phase 2 trial done at 32 centres across Europe in patients with locally diagnosed grade II or III glioma without 1p/19q co-deletion, with a first and contrast-enhancing recurrence after initial radiotherapy or chemotherapy, or both. Previous chemotherapy must have been stopped at least 6 months before enrolment and radiotherapy must have been stopped at least 3 months before enrolment. Random group assignment was done electronically through the European Organisation for Research and Treatment of Cancer web-based system, stratified by a minimisation procedure using institution, initial histology (WHO grade II vs III), WHO performance status (0 or 1 vs 2), and previous treatment (radiotherapy, chemotherapy, or both). Patients were assigned to receive either temozolomide (150-200 mg/m, orally) monotherapy on days 1-5 every 4 weeks for a maximum of 12 cycles, or the same temozolomide regimen in combination with bevacizumab (10 mg/kg, intravenously) every 2 weeks until progression. The primary endpoint was overall survival at 12 months in the per-protocol population. Safety analyses were done in all patients who started their allocated treatment. The study is registered at EudraCT (2009-017422-39) and ClinicalTrials.gov (NCT01164189), and is complete. FINDINGS Between Feb 8, 2011, and July 31, 2015, 155 patients were enrolled and randomly assigned to receive either monotherapy (n=77) or combination therapy (n=78). Overall survival in the per-protocol population at 12 months was achieved by 44 (61% [80% CI 53-69]) of 72 patients in the temozolomide group and 38 (55% [47-69]) of 69 in the combination group. The most frequent toxicity was haematological: 17 (23%) of 75 patients in the monotherapy group and 25 (33%) of 76 in the combination group developed grade 3 or 4 haematological toxicity. Other than haematological toxicities, the most common adverse events were nervous system disorders (59 [79%] of 75 patients in the monotherapy group vs 65 [86%] of 76 in the combination group), fatigue (53 [70%] vs 61 [80%]), and nausea (39 [52%] vs 43 [56%]). Infections were more frequently reported in the combination group (29 [38%] of 76 patients) than in the monotherapy group (17 [23%] of 75). One treatment-related death was reported in the combination group (infection after intratumoral haemorrhage during a treatment-related grade 4 thrombocytopenia). INTERPRETATION We found no evidence of improved overall survival with bevacizumab and temozolomide combination treatment versus temozolomide monotherapy. The findings from this study provide no support for further phase 3 studies on the role of bevacizumab in this disease. FUNDING Roche Pharmaceuticals.

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Randomized phase II trial on the addition of bevacizumab to temozolomide in first recurrence 1p/19q intact WHO grade II and III astrocytoma: the EORTC TAVAREC trial.

Prof Martin J. van den Bent[1] MD, Martin Klein[2] PhD, Prof Marion Smits[3] MD, Jaap C. Reijneveld[2] MD, Pim J. French[1] PhD, Prof Paul Clement[4] MD, Filip .Y.F. de Vos[5] MD, Antje Wick[6] MD, Paul J. Mulholland[7] MD, MD, Prof Martin J.B. Taphoorn[8] MD, Joanne Lewis[9] MD, Prof Michael Weller[10] MD, Prof Olivier L Chinot[11] MD, Prof Johan M. Kros[12] MD, Iris de Heer[1] Tina Verschuere[13], Corneel Coens[13] MSc, Vassilis. Golfinopoulos[13]MD, Thierry Gorlia[13] PhD, Ahmed Idbaih[14] MD

[1]The Brain Tumor Center at Erasmus MC Cancer Institute, Rotterdam, the Netherlands;

[2]Brain Tumor Center and Department of Medical Psychology, VU University Medical Center, Amsterdam, the Netherlands

[3]Dept of Radiology and Nuclear Medicine, Erasmus MC, Rotterdam the Netherlands,

[4] Department of Oncology, KU Leuven and Department of General Medical Oncology, UZ Leuven, Leuven Cancer Institute

[6] Dept Medical Oncology, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands

[6] Neurology Clinic, University of Heidelberg, National Center for Tumor Diseases Heidelberg, Germany

[7] Dept Medical Oncology, UCLH, London, United Kingdom

[8] Dept Neurology, MC Haaglanden, the Hague, the Netherlands

[9] Freeman Hospital, Newcastle, United Kingdom,

[10] Department of Neurology and Brain Tumor Center, University Hospital and University of Zurich, Zurich, Switzerland

[11] Neuro-Oncology division, Aix-Marseille University, AP-HM, Marseille, France

[12] Dept Pathology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands

[13] EORTC Headquarters, Brussels Belgium

[14] Sorbonne Université, ICM, Inserm, CNRS, AP-HP, Hôpital Pitié-Salpêtrière, Service de Neurologie 2-Mazarin, F-75013 Pars, France

Corresponding author:

M J van den Bent
The Brain Tumor Center at Erasmus MC Cancer Institute
Dr Molewaterplein 40
3015 GD RotterdamThe Netherlands
Tel +31 10 7041415
Fax +31 10 7041031
e-mail m.vandenbent@erasmusmc.nl

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Abstract

Background: Bevacizumab is frequently used in recurrent WHO grade II and III glioma, but this use is without evidence from randomized trials. We evaluated the use of bevacizumab in grade II and III glioma without 1p/19q co-deletion at first recurrence.

Methods: The TAVAREC trial (NCT01164189) is a randomized, open label phase II study in locally diagnosed 1p/19q non-codeleted grade II or III glioma, with a first and contrast-enhancing recurrence after initial radiotherapy and/or chemotherapy. Patients were stratified by a minimization procedure using institution, initial histology (grade II or III), WHO performance status (0 or 1 versus 2) and prior treatment (radiotherapy, chemotherapy, both). Patients were electronically randomized through the EORTC web-based system. Prior chemotherapy was allowed provided patients were at least 6 months off treatment. Patients were treated with either 150-200mg/m^2 temozolomide day 1-5 every 4 weeks for a maximum of twelve cycles, or with the same temozolomide regimen in combination with bevacizumab 10 mg/kg every 2 weeks until progression. Response, Health Related Quality of Life (HRQoL, using the EORTC QOL C30/BN20 questionnaire) and neurocognitive functioning (NCF) using a standardized test battery with Hopkins Verbal Learning, Trail Making test A/B and Controlled Oral Word Association were evaluated every 3 months. Primary endpoint was the Overall Survival (OS) rate at 12 months (OS12) in the intent-to-treat population. Tumor samples were centrally analyzed for *MGMT* status (Illumina Infinium Methylation EPIC BeadChip) and *IDH1/2* hotspot mutations. This report represents the full analysis of the study.

Findings: Between 8/2/2011 and 31/7/2015, 155 patients were randomized; median age was 44 years, 101 of 131 (77%) tested tumors showed an IDH mutation (*IDHmt)*, 27% of patients had received prior chemotherapy. OS12 was 61% in the temozolomide arm and 55% in the combination arm, with overlapping OS and progression free survival (PFS) Kaplan Meier curves. The most frequent toxicity was hematological, 17 (23%) patients in the monotherapy arm and 25 (33%) in the

combination arm developed grade 3 or 4 hematological toxicity. Infections were more frequently reported in the bevacizumab arm 29:76 versus 17:75 in the control arm). One treatment related death was reported in the combination arm (intratumoral hemorrhage).

Conclusions: In this randomized phase II in recurrent grade II and III 1p/19q intact gliomas no evidence was observed of improved OS, PFS, neurocognitive functioning or quality of life of the the addition of bevacizumab to temozolomide; regardless of IDH mutational status. This study provides no support for further phase III studies to the role of bevacizumab in this disease.

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Research in Context

Evidence before this study: Uncontrolled studies on recurrent glioblastoma reported high response rates and progression free survival rates to bevacizumab, and suggested improved overall survival. Prior to the study, a Pubmed literature search was conducted with the search parameters bevacizumab, astrocytoma and oligodendroglioma ;within the time interval January 2007 (initial reports on glioblastoma) and August 2009 (drafting of the protocol). Only uncontrolled retrospective reports on the use of bevacizumab in recurrent grade III tumors were identified, that suggested similar response rates in comparison to glioblastoma. No properly controlled studies are available on bevacizumab in grade II and III glioma.

Added value of this study. This study shows that adding bevacizumab to temozolomide in patients with recurrent grade II and III 1p/19q intact anaplastic glioma does not improve outcome, surprisingly also not with respect to response rate, progression free survival, quality of life or cognition.

Implications of all the available evidence. There is no role for adding bevacizumab to temozolomide in recurrent 1p/19q intact grade II and III glioma. With a more broader perspective, the now available trial results show that adding bevacizumab to standard of care does not improve overall survival in gliomas regardless of grade.

Introduction

Currently, the standard of care of newly diagnosed WHO grade II and III glioma consists of surgery as extensive as safely possible, followed by combinations of chemotherapy and radiotherapy once post-operative treatment is indicated.(1) Despite the much better prognosis of grade II and grade III glioma patients in comparison to glioblastoma patients, following initial treatment eventually all grade II and III glioma patients will relapse. At that time prognosis is poor, especially in tumors that show evidence of increased grade of malignancy and for many patients chemotherapy is the only remaining treatment option. Studies show that 40-60% of these patients respond to chemotherapy, with patients with combined 1p/19q loss responding more frequently and with longer duration.(2-5) At the time of relapse, these tumors often show an increased growth rate and the development of enhancement and edema. Histopathologically, these tumors present as glioblastoma showing endothelial proliferation and necrosis. Thus, at this disease stage angiogenesis plays a role, suggesting a potential role for angiogenesis inhibitors. Following the initial favorable observations on the use of bevacizumab in recurrent glioblastoma with reports that described high clinical and radiological response rates and promising progression free survival (PFS) rates, several retrospective and uncontrolled reports reported on bevacizumab in recurrent grade II and III glioma.(6-9) Because of these favorable observations, we decided to evaluate the use of bevacizumab in recurrent grade II and grade III glioma in a controlled study. In view of occurrence of pseudo-responses in bevacizumab treated glioblastoma questioning the usefulness of PFS we selected overall survival (OS) at 12 months as the primary endpoint.(10-12) To avoid a too heterogeneous patient population we included only patients without 1p/19q co-deletion. At the time of study initiation the prognostic role of IDH mutations and especially the worse prognosis of IDH wild type tumors was not fully clear.(13;14) Once the pivotal role of IDH mutations in the genesis of grade II and III glioma was

established, the protocol was amended to allow a prospectively defined subgroup analysis based on IDH mutational status.

Material and methods

Study design and participants

The TAVAREC study was designed as a two-arm open-label randomized multicenter study, to assess the activity of bevacizumab in combination with temozolomide and of temozolomide alone. Eligible were patients 18 years of age or older and with a WHO performance status 0-2, with a first recurrence of a locally diagnosed grade II or grade III glioma according to the WHO 2007 glioma classification at first diagnosis and without 1p/19q co-deletion, following radiotherapy with or without chemotherapy and relapsing more than three months after the end of radiotherapy. High dose radiotherapy (over 65 Gy) was not allowed unless the recurrence was histologically proven. Only procarbazine/CCNU/vincristine (PCV) or temozolomide were allowed as prior chemotherapy and patients needed to be more than 6 months off chemotherapy before progression. Prior treatment with anti-angiogenic treatments was not allowed. Surgery at the time of the recurrence was allowed, in which case residual and measurable disease after surgery was not required but histology must have confirmed the presence of tumor . Non-operated patients needed to have an enhancing recurrence with bi-dimensionally measurable disease (minimal diameters enhancing lesion of 10 mm) on the MRI scan, with stable or decreasing dose of steroids prior for 7 days to the baseline MR scan. Patients needed to have adequate hematological, renal and hepatic function. No other diseases interfering with follow-up including other malignancies were allowed, except for any previous malignancy which was treated with curative intent more than 5 years prior to randomization, and except for adequately controlled limited basal cell carcinoma of the skin, squamous carcinoma of the skin or carcinoma in situ of the cervix. Other exclusion criteria included presence of cardiovascular disorders, significant vascular disease within 6 months prior to randomization, prior history of hypertensive crisis or hypertensive encephalopathy, inadequately

controlled hypertension (defined as systolic blood pressure >150 mm Hg and/or diastolic blood pressure >100 mm Hg); any thrombotic or hemorrhagic event, a history of active gastroduodenal ulcer(s) or a history of abdominal fistula as well as non-gastointestinal fistula, gastrointestinal perforation or intra-abdominal abscess within the 6 months prior to inclusion.

Randomisation and masking

Patients were randomized and stratified by a minimization procedure based on the variance method with semi-random assignment as implemented by Freedman and White (1976).(15) In order to reduce treatment allocation predictability, a random allocation component was included in order to ensure an additional 15% of completely random assignments. Stratification factors were institution, initial histology (WHO grade II v ersus grade III), WHO performance status (0, 1 vs 2) and prior treatment (radiotherapy alone, temozolomide or PCV alone, vs combined chemo-irradiation with temozolomide. Patients were registered by the treating institutions and electronically randomized through the EORTC web-based ORTA system (http://www.eortc.org/investigators/). An A'Hern one stage (one-sided) testing procedure was used, assuming an OS 12 of 50% was inadequate while 65% warranting further exploration. With alfa and beta set at 0.10, with a 1:1 randomization, the required sample size was 72 eligible patients in each treatment arm for a total of 144 eligible patients. The decision rule for activity was to be performed amongst the first 72 eligible patients enrolled in the bevacizumab – temozolomide arm and the temozolomide alone arm separately (requiring 42 patients or more to be alive at 12 months to call a treatment a ‘success’).

Procedures

Patients were randomized to a) temozolomide 200 mg/m2 on day 1-5 every 4 weeks for a maximum of 12 cycles, with patients having received prior chemotherapy starting at 150 mg/m2 with dose escalation to 200 mg/m2 in case of no or minimal toxicity; or to b) the same temozolomide regimen

combined with 10 mg/kg bevacizumab intravenously every 2 weeks until progression. Treatment was discontinued at progression, unacceptable toxicity or patient refusal. Dose reductions were made as described elsewhere. (16;17) One treatment cycle was defined as a period of 4 weeks.

The baseline evaluation included a standardized MRI protocol (consisting of T2-weighted and pre- and post-contrast T1-weighted imaging), a HRQoL questionnaire (the generic EORTC QLQC30 questionnaire in combination with the BN20 brain-cancer specific module), neurocognitive testing (using a standardized psychometric assessment: Hopkins Verbal Learning Test-Revised (HVLT-R; free recall, delayed recall and delayed recognition); Trail Making Test (A and B) and Controlled Oral Word Association (COWA) as described elsewhere)(18), full clinical and neurological evaluation, ECG, as well as complete blood count, blood chemistry and urinanalysis. Bevacizumab treated patients were evaluated for vital signs, adverse events, hematology and urine dipstick exam every 2 weeks. All patients were evaluated every 4 weeks for vital signs, adverse events, and blood examinations. Every 3 months, neurological evaluation, MRI scanning, HRQoL evaluation and neurocognitive testing was performed.

Response assessment was done according to the RANO criteria.(11) In case of equivocal PD (target or non-target), treatment could continue until the next assessment, but if PD was confirmed at the next follow-up, the earlier date was used as the date of progression. All decisions on the assessment and interpretation of disease status (including 1p/19q assessment) were done locally, with preplanned central review afterwards.

At the time of study analysis central pathology review and molecular testing was performed (JMK, PF). Assessment of MGMT promoter methylation status was done using the Infinium MethylationEPIC BeadChip (Illumina, San Diego, Ca). .(19;20) The MGMT methylation status was determined by the MGMT-STP27 model using the mgmtstp27 R package (github.com) .(21) IDH1 and 2 mutations were determined using Sanger sequencing. In case of inconclusive sequencing results (e.g. due to poor DNA quality or insufficient quantity) we performed immunohistochemistry using

IDH1R132H-specific antibodies (Dianova, Germany, see Capper, ANP 2009). Positive staining on immunohistochemistry was scored as IDHmt, negative staining was scored as indeterminate IDH status as other, non-R132H-mutations, may be present.

Outcomes

The primary endpoint was probability of survival at 12 months (OS12. Secondary endpoints were best overall response, objective (partial PR and complete CR) response rate and duration of response, progression free survival (PFS) distribution and at 6 and 12 months; overall survival (OS) distribution and at 24 months (Kaplan Meier Estimates); safety profile; and patient oriented criteria: clinical/neurological deterioration free survival, steroid use, quality of life (by patients and caregivers) and development of cognitive deterioration..

Statistical analysis

OS was calculated from the date of randomization to the date of death from any cause. Patients still alive or lost to follow-up were censored at last follow-up visit date. The percentage of patients alive at 1 year is presented with binomial 80% confidence intervals (CI [,]). Both response and PFS were defined according to RANO criteria.(11)The objective response rates and other rates are reported with binomial 95% CI (,). PFS was defined as the time from randomization to the date of first progression or death, whichever came first. Patients were censored at the last follow-up visit without evidence of progression or at the date of starting a new anti-tumoral therapy before progression.

The Kaplan Meier technique was used to compute estimate of OS and PFS with 95% CI (,)The Cox proportional hazard model was used in multivariable analysis to assess the treatment effect adjusted

by the stratification factor at randomization (except institution), to infere the treatment by factor interaction term in predictive factor analysis and to identify independent prognostic factors. The hazard ratios were presented with 95% CI (,). For prognostic model building, baseline clinical and molecular factors were jointly screened. Stepwise selection method was used at 5 % significance and the bootstrap technique was employed to estimate the probability of inclusion of factors in the Cox model. A probability of inclusion of 60% or more was considered appropriate. Models discrimination was measured by the Harrel's C-index, a C-index larger than 60% was considered a minimum.(22;23)

Relative dose intensity (RDI) was calculated as the administered dose per time as delivered divided by the planned dose per planned time of delivery. Primary efficacy analyses were performed in the per protocol population defined as all eligible patients who started their allocated treatment. No direct comparison was performed in this population. As sensitivity analysis, formal comparisons of PFS and OS were performed in the intent to treat population. RDI and safety analyses were performed in the safety population defined as all patients who started allocated treatment. For all statistical analyses, SAS version 9.4 (Copyright (c) 2002-2012 by SAS Institute Inc., Cary, NC, USA. All Rights Reserved) was used.

HRQoL assessment: HRQoL data were scored according to the algorithm described in the EORTC scoring manual.(24) Herein are responses aggregated and transformed into a linear scale that ranged from 0 to 100, in which a higher score represented a higher level of functioning (function scales) or a higher level of symptoms (symptom scales). If at least half of the items in the scale were completed, the scale score was calculated with only those items for which values existed. A change was considered as clinically relevant when it was 10 points or more.The HRQoL scales endpoints that were preselected for this study are global health status, self-perceived cognitive functioning and pain; with the global health status scale as the primary HRQoL outcome for this study.

Neuro-cognitive assessment: For each of the 6 standardized psychometric test outcomes, for each patient and for each time-point, the reported test scores were converted to raw scores. (18) For each

time-point, the tests raw scores were normalized into z-scores using at baseline the patient's age (HVLT-R and Trail Making Test) or gender (COWA). The change from baseline in z-scores s calculated as z-score at post-baseline time-point minus z-score at baseline. The HVLT-R test scores were adjusted according to the patient's age. The score at the last assessment after baseline with 60% compliance was computed and compared between treatment arms by Wilcoxon rank sum test. To account for multiplicity the Hochberg Step Up Procedure was applied at an overall 5 % significance. The Reliable Change Index (RCI) was calculated for each test in order to define neurocognitive failure. This was defined for each of the 6 NCF test outcomes as a change in a raw score that exceeds the RCI calculated as ±1.64 (SEdiff), where SEdiff = [2SEM2]1/2 and SEM = SD1[1-rxy]1/2.

Neurological Deterioration was defined as a decrease in WHO performance status: for patients with baseline WHO performance status 0 or 1 deterioration to WHO performance status 2 or worse for which no other explanation is present, and which is maintained for at least 3 weeks; for patients with baseline WHO performance status 2 deterioration to WHO performance status 3 or worse for which no other explanation is present and which is maintained for at least 3 weeks. Adverse events were scored according to the NCI-CTCAE version 4 criteria.Central MR assessment: All MRI were centrally reviewed centrally according to the RANO criteria by either MvdB or MS, the reviewers were blinded to treatment allocation. Images were coded and collected centrally at EORTC HQ, and then transferred on a hard disk for review.

Organization of the trial, role of the funding source

The trial was developed by the principal investigator (MvdB) in collaboration with the leading investigators for neuroimaging (MS), molecular analysis (PF), neurocognition (MK) and health-related quality of life (MT, JR) as well as the EORTC Headquarters (TG, CC). All data have been reviewed by

EORTC Headquarter staff and MvdB, PF, JR, MT and MK where appropriate. Statistical analyses were performed by TG and CC. Translational research and molecular marker evaluation was coordinated by PF. Central imaging review was conducted by MS and MvdB. The corresponding author had full access to all of the data and the final responsibility to submit for publication. The trial sponsor was the EORTC. The trial was supported by an unrestricted educational grant and free bevacizumab supply by Hoffmann La Roche. The drug manufacturer was not involved in trial design or analysis. The study was registered at EudraCT# 2009-017422-39 and ClinicalTrials.gov NCT01164189. The protocol was approved by the ethics committees and competent authorities of all participating centers and countries. All patients gave written informed consent for trial participation, pathology review and molecular testing. The full study protocol can be reviewed at h□p://www.eortc.be/services/doc/protocols/26091v2.0.pdf

Results

Between 8/2/2011 and study closure on 31/7/2015 for enrollment completion, 155 patients were randomized; the final database lock was on 10/01/2017. Median age was 44 years, 101 (65%) of 131 tested tumors showed an IDHmt, 42 (27%) of patients had received prior chemotherapy. Patient characteristics were well balanced between treatment arms (table 1); figure 1 shows the CONSORT diagram of this study. At review, 12 patients (8 in the combination arm) were considered to not fully meet the entry criteria (inadequate baseline MR imaging (7); hypertension (1); no target lesion (3); and 2nd recurrence (1)). At central pathology review of tissue from the first diagnosis, 11 patients in both arms were considered to have had a glioblastoma. Of those 22 patients, 10 were IDHmt and 2 were IDH status undetermined. Four patients never started treatment. The median number of temozolomide cycles in the temozolomide monotherapy arm was 7 and in the combination arm 8. The median number of bevacizumab cycles (4 weeks) in the combination arm was 8. The

temozolomide RDI in the single agent arm was 98%, in the combination arm 91%. The RDI of bevacizumab was 94%. In 88 (57%) of patients, temozolomide was discontinued because of progression, in 34 (22%) because of the completion of 12 cycles and in 16 (10%) for toxicity . Bevacizumab was discontinued in 49 (63%) patients for progression, in 12 (15%) for toxicity, in 10 for other reasons; treatment was ongoing in 5 patients at the time of database lock.

Safety and tolerability

In the safety population, 37 of the 75 (49%) patients the monotherapy arm and 44 of the 76 (58%) patients in the combination arm had at least one dosage of temozolomide delayed. Forty-two patients (55%) had at least one cycle of bevacizumab delayed. The most frequent toxicity was hematological: 17 (23%) patients in the monotherapy arm and 25 (33%) in the combination arm developed grade 3 or 4 hematological toxicity (table 2)). In 17 patients (23%) treated with temozolomide alone and in 44 patients (58%) in the combination arm grade 3 of 4 adverse events were reported. Table 3 presen t other grade 1-2 adverse events occurring in ≥ 10% of patients and grade 3, 4 and 5 adverse events. There were 8 serious adverse events in the monotherapy arm (hematological: 2, hematological complicated by infection: 2, seizures:2, infection:1) and 39 in the combination arm (hematological:6, hematological complicated by infection: 2, seizures: 5, pulomonary embolism: 3, allergic reactions: 4, infections: 7). One patient in the combination arm died from a pulmonary infection after suffering from an intratumoral bleed during a related grade IV thrombocytopenia.

Outcome: survival analysis

At the time of analysis median follow-up was 28 months (interquartile range: 20.6 months), 135 patients (87%) had progressed and 24 patients (15%) were still alive. At one year 44 of the 72 eligible temozolomide patients (61% [53,69]) and 38 of the 69 (55% [47,69]) eligible combination arm

patients were alive. Median survival in this group was 14.8 month (12.9,16.9) in the temozolomide arm and 12.9 months (10.6,16.3) in the combination arm; after 2 years respectively 25.2% (14.9,36.9) and 23.5% (13.7,34.8) of patients were still alive. Median progression free survival in the eligible patient population was 6.3 months (5.5,8.5) in the temozolomide arm and 5.9 (5.6,8.2)in the combination arm. In temozolomide arm, the 6 mo NDFS was 80.0% (67.9, 87.9) and the median not reached. In the combination arm, the 6 mo NDFS was 82.5% (70.6, 89.9) and median 11.3 mo (8.8 , 22.1). Table 4 and figure 2 a and b present the Kaplan Meier analysis of PFS and OS in the intent to treat population. A total of 141 patients were evaluable for response (temozolomide arm: 72; temozolomide plus bevacizumab: 69). In the temozolomide only arm 32 of 77 patients had an objective response rate by central review (44.4%; 32.7,56.6) and 34 of 69 patients (52.2%; 39.8,64.4) in the combination arm. The duration of response was similar in both arms (temozolomide arm: 5.7 months, 95% CI [3.0, 8.6]; temozolomide plus bevacizumab 5.6 months, 95% CI [3.0, 5.9]. In the Cox model, the treatment effect was not statistically significant (HR 1.11(0.77-1.60), p=0.59), only the performance status at baseline was associated with outcome (PS 2: HR 3.86, [2.22, 6.72, p<0.0001] Prior chemotherapy (data not shown) and grade (HR 1.31(0.89,1.92), p=0.17) did not impact OS.

In the supplementary files page 2 - 4 treatment after progression following protocol therapy is shown. In the temozolomide arm 50 (79%) patients received some type of chemotherapy and 22 (33%) bevacizumab; in the combination arm 41 (59%) received some type of chemotherapy and 12 (17%) continued or were retreated with bevacizumab.

Outcome: analyses of neurocognitive functioning and HRQoL

At baseline 142:155 patients were assessed with neurocognitive testing (compliance 92% (). With 25 of 40 progression free tested patients tested at week 60 , the compliance with the neurocognitive testing remained above 60% up to that week (supplementary file table 2, page 5). There was no difference in compliance between both study arms at any time points. Neither at the last test available before or at week 60 or at longitudinal analysis during follow-up statistically significant

differences were observed between the study arms in any of the six test outcomes (Supplemental file fig 1 page 7: trail making B).

At baseline HRQoL forms were received from 145 patients (94%), with 26 of 40 progression free tested patients at week 60 the compliance with HRQoL forms remained above 60% up to that time point (supplementary table 2, page 5). There was no significant difference in compliance between both study arms. The global health status was similar in both arms throughout the follow-up (p = 0.26; figure 3), self-reported neurocognitive functioning and the pain scale also showed no difference between the two treatment arms (suppl file figure 2 and 3 page 8, 9)

Molecular analysis

In 125 patients sufficient material was available for methylation arrays. There was a trend towards worse OS in the patients with material available for this analysis compared to the 30 without material for analysis (p = 0.07). Four patients were diagnosed as 1p/19q co-deleted by methylation array (two in each study arm), they had been locally diagnosed as non-codeleted, all had oligodendroglial histology. Of these 125 cases, 92 had IDH mutations and 26 were IDHwt (7 missing), 91 were considered MGMT promoter methylated. Of the 92 IDHmt tumors with available MGMT status, 77 (83.6%) were considered MGMT methylated. The presence or absence of an IDH mutation was not associated with any trend towards benefit of adding bevacizumab (IDH mutated (HR 0.94, (0.59, 1.49); IDHwt population HR 1.46, (0.68, 3.07); interaction test p=0.33). The 6 mo PFS in MGMT promoter methylated patients was 59.3%(48.5,68.6), in unmethylated 29.4% (15.4,45.0), HR 0.47 (0.31, 0.72)

Prognostic and predictive factor analysis

In the absence of an improved outcome in the combination arm, in an exploratory analysis we analysed both treatment arms together. Sex, age, surgery at recurrence, prior chemotherapy, frontal

involvement, tumor grade at initial surgery did not have any prognostic value. Median survival in initially as grade II diagnosed tumors was similar to initially grade III tumors: 14.5 months (12.6-16.6) versus 14.8 months (11.3-17.8), p = 0.64, supplemental file page 10 figure 4a). In contrast, MGMT status, IDH status (median OS IDHwt patients 10.1 months versus IDHmt patients 15.2 months, p < 0.001; supplemental page 10 fig 4b), performance status, use of steroids, number of target lesions, size of the lesion at recurrence and time since initial surgery had statistically significant correlations with OS. In multivariate analysis including both MGMT and IDH, both were independent factors associated with OS (IDHmt: HR 0.50 0.31, 0.80) p = 0.004; and MGMTmeth HR 0.48 (0.31, 0.76), p = 0.002); bootstrapping included MGMT in 77% and IDHmt in 58% of the models. In multivariate analysis using both clinical and molecular factors, lesion maximum diameter (p=0.0002), performance status 2 (p<0.0001), MGMT (p=0.002) and IDH status (p=0.003) were identified as factors of independent prognostic significance. (C-index 68%). All were confirmed with bootstrapping (inclusion probability MGMT 80%, IDH 72%). Corticosteroids use was predictive for benefit to temozolomide monotherapy (interaction test, p = 0.014). In exploratory analysis, no subgroup benefitting from adding bevacizumab to temozolomide could be identified (supplemental files figure 5 page 10-14).

Discussion

Similar to the initial trials in glioblastoma, initial uncontrolled series suggested a benefit of bevacizumab in relapsing astrocytoma and oligodendroglioma. (6-9) This study on 155 recurrent WHO grade II and III glioma patients without 1p/19q co-deletion treated at first and enhancing recurrence however did not show any indication of an improvement in outcome with the addition of bevacizumab to temozolomide. Moreover, we did also not see an improved response rate in the combination arm, and taken together this appears to exclude a relevant role of VEGF signaling in this disease. We selected patients with an enhancing recurrence, in which neo-angiogenesis is more likely

to have developed and histology is likely to resemble glioblastoma. Whether there is still a role for bevacizumab as an anti-edema agent in late-stage disease and in palliative setting of relapsing astrocytoma patients cannot be answered by this trial, but a significant anti-tumor effect can be ruled out. At the time of analysis 12 patients were judged ineligible at medical review, predominantly because of absence of measurable disease and 4 patients did not start the allocated treatment. Nonetheless, even if 3 more eligible patients would have been present in the combination arm and they would all have survived at one year, the primary endpoint would still not have been met. Moreover, the PFS and OS analysis in both the eligible patient population and the intent to treat patient population failed to show any evidence of improved outcome in the combination arm when considering the temozolomide monotherapy arm.

Bevacizumab has now been registered for use in glioblastoma for its beneficial effect on PFS and the reduction of steroid use. The results of the TAVAREC trial are different: no PFS improvement was observed which raises the question whether VEGF signaling is less relevant in dedifferentiated grade II and III glioma as compared to glioblastoma. Moreover, despite the anti-edema effects of bevacizumab in glioblastoma and presumed effects on neurological functioning of patients, no difference was observed in the quality of life nor in the neurocognitive functioning of patients. We have no evidence that bevacizumab improved the functioning of patients, and since in this study PFS was similar in both arms there is no impact of duration of treatment on the analysis allowing a proper assessment of the impact of bevacizumab on patient functioning. One difference between this trial and the trials on recurrent glioblastoma is the less frequent use of steroids at baseline in the anaplastic glioma patient population at first recurrence.

The most relevant limitation of this study is that it is a randomized phase II study, not powered for formal comparison. It is however still the largest randomized trial on recurrent astrocytoma since the pivotal temozolomide randomized phase II registration trial.(2) Also, the treatment approach to this disease is variable, with different strategies early on in the disease which impact treatment at

progression. To have a more homogeneous patient population we did not allow 1p/19q co-deleted tumors, although four co-deleted tumors were identified afterwards as part of the molecular studies. Importantly, the current study confirmed our expectation at the time of the study design that tumor grade (grade II or grade III) at initial diagnosis does not impact OS once an enhancing recurrence develops, but clearly IDH mutational status does. Since IDHwt and IDHmt glioma present different diseases, they should be kept separate in future studies, but tumor grade (grade II versus grade III) is irrelevant in this setting. We were in particular interested whether we could identify molecular factors that would allow the selection of patients that were unlikely to benefit from temozolomide. As expected, MGMT promoter methylation and IDH status were highly correlated and both were correlated with survival. In glioblastoma, the absence of MGMT promoter methylation has been found to be associated with a very poor outcome to 2nd line chemotherapy.(17;25) In this dataset, the 6 mo PFS was still 29% in patients with MGMT unmethylated tumors, which implies that in these tumors absence of MGMT methylation cannot be used as a reliable criterion for a decision to withhold chemotherapy at the time of progression.

To conclude, in WHO grade II and III 1p/19q intact relapsing glioma, the addition of bevacizumab to temozolomide does not improve survival or quality of survival. Future trials on 1p/19q intact glioma can combine grade II and III tumors, but should distinguish between IDHmt and IDHwt tumors.

For an overview of participating sites, principal investigator and accrual per site, see supplemental file table 3, page 6.

Author contribution section

The literature search was done by MJvdB, MK, MS, JCR and AI. The study was designed by MJvdB, TG, AI, MK, JCR, MJBT, MS, PF. The data were collected by all. The data were analysed MJvdB, MK, MS,

JCR, PF, CC, VG, TG, AI. The manuscript drafts were written by MJvdB, MK, JCR, PF, TG. All authors approved of the final version of the manuscript.

Declaration of interests

Dr. van den Bent reports grants and personal fees from Roche, during the conduct of the study; personal fees from Celgene, grants and personal fees from Abbvie, personal fees from Actelion, personal fees from Celldex, personal fees from BMS, personal fees from Daiichi Sankyo, personal fees from MSD, outside the submitted work; Dr. Smits reports other from Parexel Ltd, outside the submitted work; Dr. Reijneveld reports other from Roche Nederland NV, outside the submitted work; Dr Clement reports other from Bristol Myers Squibb Belgium N.V., Merck NV, Vifor Pharma Belgium NV, Astrazeneca UK limited, Abbvie, Leo Pharma NV, MSD Belgium BVBA outside the submitted work; Dr. Taphoorn reports other from Hoffmann La Roche, outside the submitted work; Dr. Weller reports grants from Actelion, grants from Acceleron, grants from Piqur, grants from OGD2, grants and personal fees from Roche, grants and personal fees from Merck (MSD), grants from Merck (EMD) , personal fees from Celldex, personal fees from Celgene, grants and personal fees from Tragara, grants and personal fees from Abbvie, grants and personal fees from Novocure, personal fees from Orbus, personal fees from Tocagen, outside the submitted work; Dr. Chinot reports grants, personal fees and non-financial support from Roche, personal fees and non-financial support from BMS, personal fees and non-financial support from Abbvie, personal fees from Immatics, personal fees from Celdex, personal fees and non-financial support from Servier, personal fees from Ipsen, outside the submitted work; Dr. IDBAIH reports grants from Fondation ARC, other from Hoffmann-La Roche, other from Carthera (juin 2017), personal fees from BMS (nov 2015), personal fees from Hoffmann-La Roche (dec 2015), personal fees from Cipla (dec 2015) (certified continuing education), outside the submitted work. The other authors declared no conflicts of interest.

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Figure 1. CONSORT flow diagram

Figure 2A: Progression Free survival in the intent to treat population

Figure 2B. Overall survival in the intent to treat population

Supplemental file 1. Forest plot of known and potential major clinical and molecular prognostic factors.

Table 1 Baseline characteristics at randomization

	TMZ (N=77) N (%)	TMZ+Bv (N=78) N (%)	N (%)
Sex: male	45 (58.4)	57 (73.1)	102 (65.8)
Median age at randomization (years)	43.1	44.6	43.3
Prior chemotherapy given			
no	56 (72.7)	57 (73.1)	113 (72.9)
TMZ (Concomitant and/or Adjuvant = one line)	21 (27.3)	16 (20.5)	37 (23.9)
PCV	0 (0.0)	5 (6.4)	5 (3.2)
WHO grade at first (local) diagnosis			
Grade II	40 (51.9)	43 (55.1)	83 (53.5)
Grade III	36 (46.8)	34 (43.6)	70 (45.2)
Missing	1 (1.3)	1 (1.3)	(1.3)
WHO performance status			
0	34 (44.2)	31 (39.7)	65 (41.9)
1	35 (45.5)	38 (48.7)	73 (47.1)
2	8 (10.4)	9 (11.5)	17 (11.0)
Prior irradiation given			
no	2 (2.6)	5 (6.4)	7 (4.5)
Yes	75 (97.4)	73 (93.6)	148 (95.5)
Surgery at the time of progression			
yes	22 (28.6)	24 (30.8)	46 (29.7)
Corticosteroids intake			
Yes	27 (35.1)	22 (28.2)	49 (31.6)
Time since last radiotherapy (months)			
Median	29.3	28.1	28.7
Range	3.6 - 177.1	4.2 - 239.6	3.6 - 239.6
MGMT			
Unmethylated	12 (15.6)	22 (28.2)	34 (21.9)
Methylated	51 (66.2)	40 (51.3)	91 (58.7)
Not determinable	14 (18.2)	16 (20.5)	30 (19.4)
IDH			
Wildtype	14 (18.2)	16 (20.5)	30 (19.4)
Mutated	53 (68.8)	48 (61.5)	101 (65.2)
Undetermined	10 (13.0)	14 (17.9)	24 (15.5)

Table 2. Hematological toxicity in the safety population

	Temozolomide (n = 75)			Temozolomide and Bevacizumab (n = 76)		
Hematological toxicity	Grade 2	Grade 3	Grade 4	Grade 2	Grade 3	Grade 4
ANC	6 (8%)	3 (4%)	3 (4%)	10 (13%)	6 (8%)	5 (7%)
WBC	7 (9%)	2 (3%)	1 (1%)	16 (21%)	3 (4%)	2 (3%)
platelets	8 (11%)	3 (4%)	5 (7%)	13 (17%)	6 (8%)	7 (9%)
lymphocytes	21 (28%)	3 (4%)	5 (7%)	25 (33%)	12 (16%)	1 (1%)
hemoglobin	1 (1%)	2 (3%)	0	2 (3%)	0	0

Table 3. Adverse events (worst grade)per patient adverse events per treatment arm in the safety population.

	TEMOZOLOMIDE (n = 75)				TEMOZOLOMIDE + BEVACIZUMAB (n = 76)				
ADVERSE EVENT	**Grade 0**	**Grade 1-2**	**Grade 3**	**Grade 4**	**Grade 0**	**Grade 1-2**	**Grade 3**	**Grade 4**	**Grade 5**
BIOCHEMISTRY									
SGPT/ALAT	38	32 (43%)	3 (4%)	0	39	35 (46%)	2 (3%)	0	0
HYPOCALCEMIA	63	7 (9%)	2 (3%)	0	64	4 (5%)	6 (8%)	1 (1%)	1 (1%)
HYPONATREMIA	67	6 (8%)	0	0	65	3 (4%)	8 (11%)	0	0
HYPERKALEMIA	66	2 (3%)	6 (8%)	0	50	2 (3%)	23 (30%)	0	1 (1%)
FEBRILE NEUTROPENIA	74	0	1 (1%)	0	76	0	0	0	0
VENTRICULAR DYSFUNCTION	75	0	0	0	75	0.	1(1%)	0	0
VOMITING	55	20 (27%)	0	0	55	19 (25%)	2 (3%)	0	0
CONSTIPATION	48	27 (36%)	0	0	46	30 (39%	0	0	0
DIARRHOEA	69	6 (8%)	0	0	62	12(16%)	2 (3%)	0	0
NAUSEA	36	39 (52%)	0	0	33	42 (55%)	1 (1%)	0	0
PANCREATITIS	75	0	0	0	75	0	1 (1%)	0	0
WEIGHT DECREASED	69	6 (8%)	0	0	59	16 (21%)	1 (1%)	0	0
WEIGHT INCREASED	59	16 (21%)	0	0	57	17 (22%)	2 (3%)	0	0
FATIGUE	22	52 (69%)	1 (1%)		15	54 (71%)	7 (9%)	0	0
HEPATIC FAILURE	74	0	1 (1%)	0	76	0	0	0	0
ANAPHYLACTIC REACTION	75	0	0	0	75	0	1	0	0
HYPERSENSITIVITY	72	3 (4%)	0	0	71	2 (3%)	3 (4%)	0	0
INFECTIONS (ALL)	58	15 (20%)	2 (3%)	0.	47	18 (24%)	10 (13%)	0	1 (1%)
WOUND DEHISCENCE	74	0	1	0	75	1	0	0	0
HYPERGLYCEMIA	73	1 (1%)	0	1 (1%)	75	0	1 (1%)	0	0
NERVOUS SYSTEM DISORDERS	16	49 (65%)	10 (13%)	0	11	48 (63%)	15 (20%)	2 (3%)	0
SEIZURES	42	28 (37%)	5 (7%)	0	41	27 (36%)	7 (9%)	1 (1%)	0
PROTEINURIA	75	0	0	0	68	7 (9%)	1 (1%)	0	0
COUGH	71	4 (5%)	0	0	68	8 (11%)	0.	0	0
DYSPHONIA	74	1 (1%)	0	0	67	9 (12%)	0	0	0
EPISTAXIS	74	1 (1%)	0	0	68	8 (11%)	0	0	0
RASH	69	6 (8%)	0	0	64	8 (11%)	4 (5%)	0	0
SKIN ULCER	75	0	0	0	75	0	1 (1%)	0	0
EMBOLISM	75	0	0	0	75	0	1 (1%)	0	0
HYPERTENSION	61	13 (17%)	1 (1%)	0	39	27 (36%)	10 (13%)	0	0

Table 4. Progression free and overall survival in the intent to treat population (in bold: % 12 months (mo) overall survival in the intent to treat population)

	Progression free survival			Overall survival		
	Median	6 mo	12 mo	Median	**12 mo**	24 mo
Temozolomide	6.1 mo	50.0%	30.3%	15.0 mo	**63.1%**	26.8%
temozolomide/bevacizumab	6.9 mo	53.9%	27.6	13.8 mo	**59.8%**	26.2%

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